

Assessment of Periodontal Health Status and Serum Level of Vascular Endothelial Growth Factor in Women with Breast Cancer

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Key words

Periodontal diseases, breast cancer, VEGF, angiogenesis.

Abstract

Periodontal diseases are inflammatory diseases that affect the periodontium which lead to alveolar bone and attachment loss. Breast cancer is the most common cancer in women worldwide. Expression of (VEGF) is elevated in both diseases and it is the marker of angiogenesis related to cancer development. 1. To determine the periodontal health status and the serum levels of (VEGF) in women with breast cancer in comparison to women without breast cancer. 2. To correlate between serum levels of VEGF with clinical periodontal parameters (PLI, GI, BOP, PPD, and CAL) in women with breast cancer in comparison to women without breast cancer. Eighty women with age ranged from (30-60) years old, 40 women with breast cancer and another 40 women without. Periodontal health status were taken for all participants and then they were subdivided into four groups: the (BC +CP) group which included participants with breast cancer and chronic periodontitis (n=20), the (BC+ G) group included participants with breast cancer and gingivitis (n=20), the (CP) group which included participants with chronic periodontitis only (n=20) and the (G) group which included participants with gingivitis only (n=20). Other than breast cancer, all subjects were systemically healthy females. Periodontal health status was determined by clinical periodontal examination of (PLI, GI, BOP, PPD and CAL). Blood samples were collected from each participant, serum levels of VEGF were determined by enzyme -linked immune-sorbent assay (ELISA). The results of this study showed that the median values of PLI and GI were slightly increased in BC+G group in comparison to other groups with non-significant differences, the percentages of BOP showed highly significant differences among the groups (P <0.01). The median values of PPD showed slight increase in BC+CP group than CP group with non-significant differences. Median values of CAL were increased in CP group than BC+CP group with non-significant differences. VEGF serum levels were elevated in BC+CP group than other groups with non-significant differences. Also by using Spearman's rank Correlation Coefficient, serum levels of VEGF were correlated positively with all clinical periodontal parameters. VEGF serum level could be associated with progression of both periodontal diseases and breast cancer.

Introduction:

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Periodontal diseases are bacterial infections that affect the periodontium which includes; gingiva, cementum, periodontal ligaments and alveolar bone

that supports the teeth. This inflammatory diseases result from the host response to the bacteria in dental biofilm. It may be reversible and remain confined to the gingival tissues with minimal tissue alterations which is called gingivitis or it may be irreversible and progress to further periodontal destruction with the loss of attachment and alveolar bone which is called chronic periodontitis. In addition to the presence of periodontopathogens; such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia*; genetic and environmental factors are able to enhance the susceptibility of some people in developing more severe inflammatory diseases⁽¹⁾. It has been established that the severity of periodontal diseases is dependent on a dynamic equilibrium of interactions between the microbial invasion and host immune responses⁽²⁾. Cancers represent a heterogeneous group of diseases characterized by uncontrolled growth and spread of abnormal malignant cells in the body. The disruptive behaviors of cancer cells reflect dynamic changes in their gene expression that result in disruption of normal regulatory signaling process⁽³⁾. Breast cancer is the most common type of malignancy registered in the cancer records of almost all countries within the Eastern Mediterranean Region. Breast cancer is originating from breast tissues, mostly from the inner lining of lobular ducts that supply the ducts with milk. Malignancy of the breast is one of the most life threatening diseases in women globally⁽⁴⁾. Due to poor oral hygiene and gram-negative anaerobic bacterial infection, chronic periodontitis is closely associated with an increased production of reactive oxygen species that might cause damage to the host cells and tissues⁽⁵⁾. Vascular endothelial growth factor (VEGF), a glycoprotein, has attracted attention as a potential inducer of angiogenesis. It is present in periodontal tissues within endothelial cells, plasma cells, macrophages, in junctional, sulcular and gingival epithelium. In periodontitis patients, the total amount of VEGF collected from diseased sites were greater than from clinically healthy sites⁽⁶⁾. Vascular endothelial growth factor

(VEGF) is reported to be a key mediator of angiogenesis which performs a central role in both local tumor growth and distant metastasis in numerous tumors mainly breast cancer⁽⁷⁾. This study was decided to be conducted because there is not enough information about association of VEGF with both periodontal diseases and breast cancer then evaluate the serum levels of VEGF in these patients.

Materials and method:

The subjects who participated in this study were consisted of (80) women with the age range from (30 to 60) years old. The participants recruited for the study were attendants of Oncology teaching hospital/ Baghdad Medical city, or attendants to the Iraqi blood bank / Baghdad Medical city, for blood donation. Samples collection was started from October 2015 to January 2016. All participants were informed about the aims of the study by a written informed consent and it was assigned by all participants. Samples of 3ml of venous blood were withdrawn from each subject. Periodontal health status was assist through the examination of clinical periodontal parameters (plaque index PLI⁽⁸⁾, gingival index GI⁽⁹⁾, bleeding on probing BOP⁽¹⁰⁾, probing pocket depth PPD⁽¹¹⁾ and clinical attachment level CAL). forty Women with breast cancer were 40 and another 40 women without breast cancer and the sample was subdivided into four groups:

- 1. First group (BC+CP);** consisted of twenty patients with newly diagnosed breast cancer and chronic periodontitis, those patients should have at least 4 sites with periodontal pockets with depth of ≥ 4 mm with clinical attachment loss of 1-2 mm or greater⁽¹¹⁾.
- 2. Second group (BC+G);** consisted of twenty patients with newly diagnosed breast cancer and having gingivitis.
- 3. Third group (CP);** consisted of twenty patients with chronic periodontitis only without breast cancer and no history of any other systemic diseases.
- 4. Fourth group (G);** consisted of twenty patients with gingivitis only without breast

cancer and no history of any systemic diseases.

The blood samples were collected in serum separating gel tubes, allow samples to clot for 30 minutes then centrifuged for 15 minutes at 1000 x g (3500 Rpm) then serum were separated in plain tubes and stored at deep freezer (-64 °C) in plastic containers for later analysis by Enzyme Linked Immunosorbent Assay (ELISA) using kit for quantitative determination of serum levels of VEGF. The laboratory tests were done in the Teaching Laboratories of Baghdad Medical City.

Statistical analysis was done using mean, median, Min, Max, SD, SE, percentages, Kruskal-Wallis H test, Mann-Whitney U test, Chi-square test, Shapiro-Wilk test, Spearman's rank correlation coefficient test (r). Level of significance used in this study was 0.05.

Results:

The present study showed the median values of the periodontal parameters (PLI, GI, BOP, PPD, CAL) in the groups with and without breast cancer were (1.32, 1.21), (1.38, 1.29), (34, 30), (4.51, 4.45), and (3.34, 5.28) respectively (Table 1). This study showed that the median values of PLI and GI were elevated in BC+G group in comparison to other groups, with non-significant differences, the median values of PLI for BC+CP, BC+G, CP and G groups were (1.27, 1.41, 1.19, 1.27) respectively and The median values of GI for the same previously mentioned groups were (1.25, 1.45, 1.23, 1.32) respectively (Table2). The percentages of non-bleeding and bleeding sites in the group BC+CP were (71.08% and 28.92%), in group BC+G were (52.61% and 47.39%) ,in group CP were (66.88% and 33.12%) ,and in group G were (59.20% and 40.80%) respectively. By using Chi-square test, the results of BOP revealed highly significant differences among the groups (Table3). The intergroup comparison of BOP parameter among the groups was showed in (Table4). The median value for PPD of group BC+CP was (4.51), it was slightly higher than group CP which was (4.45) with no-significant differences between

the groups. The median value of CAL for BC+CP group was (3.34) and for group CP was (5.29). The median value of CAL in CP group was higher than BC+CP group (Table 5). It was clearly shown that median value of VEGF of group BC+CP which was (587.68) was the higher median value among groups. The median value of group BC+G was (345.79), group CP was (368.74), and for group G was (396.27) (Table6). There were significant differences in VEGF parameter found between BC+CP and CP groups. Also there were significant differences in VEGF parameter found between BC+CP and G groups (Table7). By using Spearman's rank Correlation Coefficient, serum levels of VEGF were correlated positively with all clinical periodontal parameters (PLI, GI, BOP, PPD and CAL) (Table8).

Discussion:

Both periodontal diseases and breast cancer are multi-factorial diseases and lead to release of many inflammatory mediators from the damaged tissues into saliva and blood. These mediators may be used for diagnosis and prediction of progression of both periodontal diseases and breast cancer⁽¹²⁾. PLI, GI median values and BOP percentages were higher in BC+G group than other groups. Dental plaque is the major etiological factor in periodontal diseases. This bacterial aggregation leads to formation of microbial bio-film and bacterial invasion which result in destruction of the periodontium thus enhance plaque accumulation on the teeth surfaces⁽¹³⁾. In addition that the women with breast cancer neglected their oral hygiene, which may be due to depressed state of mind which keeps them out from regular brushing of teeth and usage of cleaning aids⁽¹⁴⁾. The median values of PPD were slightly higher in BC+ CP group than in CP group but the median values of CAL were higher in CP group than in BC+CP group. The development of chronic periodontitis is commonly preceded by several instances of gingivitis; PPD would tend to underestimate disease prevalence because

gingival recession may reduce PPD⁽¹⁵⁾. Second, gingival inflammation may be associated with fast progression in the PPD which is an indicator of current illness status⁽¹⁵⁾, CAL parameter would tend to overestimate periodontitis prevalence since attachment loss can be due to non-inflammatory causes and CAL is an indicator of cumulative tissue destruction, including past periodontal diseases⁽¹⁵⁾. Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that induces vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. The normal function of VEGF's is to form new blood vessels during embryonic development, after injury, muscle following exercise, and new vessels to bypass blocked vessels^(16,17). VEGF is the key mediator of angiogenesis in cancer that is up-regulated by oncogene expression⁽¹⁸⁾. Increased VEGF expression in epithelial cells and endothelial cells in periodontal diseases is a very useful marker for periodontal diseases detection.

In periodontitis patients, VEGF is detected within vascular endothelial cells, neutrophils, plasma cells, and junctional, pocket and gingival epithelial tissues⁽¹⁹⁾. The results of the present study showed positive non-significant correlations between VEGF and the clinical periodontal parameters (PLI, GI, BOP, PPD and CAL) among different groups. The cause of non-significant results may be due to reduced number of subject's distribution in each group. The results of this study were in agreement with Pradeep AR et al, 2011 and Lee E et al, 2003^(20,21) who found that serum VEGF levels correlated positively with the clinical periodontal parameters (PLI, GI, BOP, PPD and CAL) and concluded that serum VEGF levels increased progressively with both chronic periodontitis and gingivitis. In conclusion, VEGF can be used as a marker of periodontitis and breast cancer so it may contribute in identification of higher risk individuals as well as lead to new therapeutic approach.

Table1: Descriptive and analytic statistics of periodontal parameters in the groups with and without breast cancer with comparison of significance.

Periodontal Parameter	Median	Mean	Min.	Max.	S.D.	X ²	p-value	Sig.
PL	1.32	1.45	1	2.10	0.38	681.5	0.25	NS
With cancer								
Without cancer	1.21	1.37	1	2.43	0.39			
GI	1.38	1.37	1	2.00	0.29	767.5	0.75	NS
With cancer								
Without cancer	1.29	1.36	1	2	0.31			
BOP	34	36.70	0	92	27.38	769.5	0.77	NS
With cancer								
Without cancer	30	36.65	0	112	30.85			
PPD	4.51	4.54	4	5.33	0.43	179	0.54	NS
With cancer								
Without cancer	4.45	4.47	4	5.21	0.37			
CAL	3.34	3.98	2.20	6.36	1.38	150.5	0.18	NS
With cancer								
Without cancer	5.28	4.75	2	7	1.76			



Table 2: Analytic statistics of median values of PLI, GI parameters for the groups with comparison of significance

Groups	PLI				GI			
	Median	X ²	p-value	Sig.	Median	X ²	p-value	Sig.
BC+CP	1.27	4.276	0.233	NS	1.25	3.731	0.292	NS
BC+G	1.41				1.45			
CP	1.19				1.23			
G	1.27				1.32			

Table 3: The distribution of the numbers and percentages of sites according to the presence or absence of BOP with comparison of significance among the groups.

Groups		Score 0	Score 1	Total	X ²	d.f.	p-value	Sig.
BC+CP	No.	1288	524	1812	163.861	3	0.000	HS
	%	71.08	28.92	100				
BC+G	No.	1048	944	1992				
	%	52.61	47.39	100				
CP	No.	1240	614	1854				
	%	66.88	33.12	100				
G	No.	1236	852	2088				
	%	59.20	40.80	100				

Table 4: The intergroup comparison of BOP parameter among the groups.

Groups	X ²	d.f.	p-value	Sig.
BC+CP vs. BC+G	136.61	1	0.000	HS
BC+CP vs. CP	7.549	1	0.006	HS
BC+CP vs. G	60.026	1	0.000	HS
BC+G vs. CP	81.162	1	0.000	HS
BC+G vs. G	17.938	1	0.000	HS
CP vs. G	24.842	1	0.000	HS

Table 5: Analytic statistics of median values of PPD and CAL parameters for BC+CP and CP groups with comparison of significance.

Groups	PPD				CAL			
	Median	X ²	p-value	Sig.	Median	X ²	p-value	Sig.
BC+CP	4.51	179	0.570	NS	3.34	150.5	0.180	NS
CP	4.45				5.29			



Table 6: Analytic statistics of median values of VEGF parameter for the groups with comparison of significance.

Groups	VEGF			
	Median	X ²	p-value	Sig.
BC+CP	587.68	6.898	0.075	NS
BC+G	345.79			
CP	368.74			
G	396.27			

Table 7: The intergroup comparison of VEGF parameter among groups.

Groups	BC+CP vs. BC+G	BC+CP vs. CP	BC+CP vs. G	BC+G vs. CP	BC+G vs. G	CP vs. G
Mann-Whitney U test	128	122.5	118	188.5	177	190
p-value	0.051	0.036	0.026	0.756	0.533	0.787
Sig.	NS	S	S	NS	NS	NS

Table 8: Spearman’s Correlation Coefficient (r) between serum levels of VEGF and periodontal parameters for each group.

Periodontal parameters	Correlations	VEGF (BC+CP)	VEGF (BC+G)	VEGF (CP)	VEGF (G)
PI	r	0.075	0.118	0.303	0.292
	p-value	0.753	0.619	0.194	0.212
	Sig.	NS	NS	NS	NS
GI	r	0.179	0.207	0.018	0.037
	p-value	0.449	0.382	0.941	0.878
	Sig.	NS	NS	NS	NS
BOP	r	0.109	0.128	0.035	0.023
	p-value	0.646	0.589	0.883	0.922
	Sig.	NS	NS	NS	NS
PPD	r	0.333		0.213	
	p-value	0.151		0.366	
	Sig.	NS		NS	
CAL	r	0.009		0.265	
	p-value	0.969		0.258	
	Sig.	NS		NS	



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